

Material Properties and Biocompatibility of Self-Crosslinkable Poly(caprolactone fumarate) copolymer as a Scaffold for Guided Tissue Regeneration

Esmail Jabbari¹, Lichun Lu¹, James A. Gruetzmacher¹, Syed Ameenuddin², Godard C. de Ruiter², Michael J. Moore¹, Bradford L. Currier¹, Robert J. Spinner², Anthony J. Windebank², Michael J. Yaszemski¹

¹*Department of Orthopedic Surgery and Department of Physiology and Biomedical Engineering,*
²*Department of Neurology, Mayo Clinic College of Medicine, Rochester, MN, 55905*

Introduction

Many clinical situations require treatment options for bone defects. Biodegradable scaffolds that can be injected and crosslinked in situ to fill these defects offer attractive additions to existing methods [1]. Although biocompatible polymers that can be injected and crosslinked in situ have been developed for bone tissue engineering, a toxic crosslinking agent such as N-vinyl pyrrolidone (NVP) or methacrylic anhydride in amounts greater than 20% by weight of the macromer is required for crosslinking [2]. We recently developed a novel semi-crystalline poly(caprolactone-fumarate) (PCLF) macromer that self-crosslinks in the absence of a crosslinking agent. This semi-crystalline copolymer has a melting point between 45-55°C depending on PCL molecular weight and degree of copolymerization. Above its melting point, the copolymer is a liquid and it can be used as an injectable matrix to fill irregularly shaped defects. As the matrix cools to physiological temperature, the copolymer self-crosslinks and hardens in-situ physically by crystallization and chemically by radical polymerization. The objective of this work was to characterize the material properties and biocompatibility of the PCLF macromer.

Methods

PCLF was synthesized by condensation polymerization of PCL with fumaryl chloride in methylene chloride with triethylamine as the catalyst. Three PCLs with number average molecular weight (Mn) of 340, 760, and 1200 Daltons were used. The polydispersity indices of the PCLs were 1.7, 1.8, and 1.8, respectively, as determined by gel permeation chromatography (GPC). The PCLs were dried under vacuum of less than 5 mm Hg at 60°C for at least 12 h before the reaction. The molar ratio of fumaryl chloride to PCL was 0.9. A typical reaction for PCL with Mn of 760 was as follows. In a three-neck reaction flask, 40 mmol of PCL was dissolved in 300 ml of methylene chloride under nitrogen atmosphere. Thirty-six mmol of fumaryl chloride and 72 mmol of triethylamine dissolved in 25 ml of methylene chloride were added dropwise to the reaction with stirring. The reaction vessel was placed in an ice bath to limit the temperature rise of the exothermic reaction. After the addition of fumaryl chloride and triethylamine, reaction was continued for 24 h under ambient conditions.

After completion of the reaction, solvent was removed by rotovaporation at ambient temperature and reduced pressure, the residue was dissolved in anhydrous ethyl acetate, and the by-product triethylamine hydrochloride salt was removed by filtration. Ethyl acetate was removed by vacuum distillation. The polymer was redissolved in methylene chloride and precipitated twice in ice cold ethyl ether. The polymer was dried in vacuum (less than 5 mmHg) at ambient temperature for at least 12 h and stored at -20°C until used.

GPC was used to determine the molecular weight and polydispersity of the PCLF macromer. Monodisperse polystyrene standards with Mn of 0.474, 6.69, 18.6, and 38 kD, and

polydispersities of less than 1.1, were used to construct the calibration curve. NMR was used to confirm the presence of the fumarate group in the macromer. Polymer solutions for NMR were prepared with deuterated chloroform (99.8 atom % Deuterated, Aldrich) at a concentration of 50 mg/ml containing 1% v/v TMS as the internal standard. FTIR was used to measure the absorption of PCLF in the IR region. A drop of PCLF in acetone solution (50 mg of PCLF in 1 ml of acetone) was placed on the CaF₂ disk and dried at ambient conditions for 30 min. It was next dried in vacuum at ambient temperature for 30 min, and finally heated to 60°C to remove any residual solvent in the film.

Scaffolds were prepared by self-crosslinking of the fumarate carbon-carbon double bonds via free radical polymerization with sodium chloride salt particles as the porogen. Benzoyl peroxide and dimethyl toluidine were used as the free radical initiator and accelerator, respectively. A typical procedure for fabrication of scaffolds was as follows. 1 g of PCLF was mixed with 5.0 g of porogen in a scintillation vial, corresponding to 75% by volume porosity. Fifty µl of initiator solution (50 mg of benzoyl peroxide in 250 µl of NVP) and 40 µl of accelerator solution (60 µl of dimethyltoluidine in 940 µl of methylene chloride) were added and mixed thoroughly. NVP, in amount less than 2% by weight of PCLF, was used to dissolve the initiator and not as a crosslinking agent. The polymerizing scaffold was transferred into a mold and pressed manually and crosslinked. After crosslinking, cylindrical specimens with diameter and length of 5 mm by 8 mm were cut. The salt was leached out by placing the scaffolds in distilled water (DW) for 3 days, during which time water changes occurred every 12 hours. The scaffolds were dried in a controlled atmosphere at ambient temperature for 1 d and in vacuum of less than 5 mmHg for at least 12 h. The disks were sterilized with ethanol overnight and washed with sterile PBS before adding to the well plate.

ASTM F813-01 was used for direct contact cell evaluation of the PCLF scaffolds. Immortalized hFOB cells were used. The cryopreserved cells were thawed and plated on polystyrene flasks in media containing DMEM/F12 and sodium bicarbonate, 10% v/v fetal bovine serum (FBS), and 150 mg of Geneticin. After plating, the suspension was incubated for 12 h in a 5% CO₂, 95% relative humidity incubator at 34°C. The cells were seeded in a 24 well plate at a density of 6.5x10⁴ cells/cm² in 300 µl of media and incubated for 48 h. Next, the disks were removed, fresh media was added to each well, and incubated for an additional 24 h. The cells were counted with a haemocytometer using a Trypan Blue stain

Results

The molecular weight of PCLF as a function of PCL molecular weight is shown in Table 1. The FC/PCL ratio was 0.9 for all formulations.

Table 1

Sample	FC/PCL Ratio	PCL		PCLF	
		M _n	PI	M _n	PI
1	0.9	340± 10	1.7± 0.1	3150± 90	2.9± 0.1
2	0.9	760± 60	1.8± 0.1	3680± 20	2.3± 0.1
3	0.9	1200± 50	1.8± 0.1	3870± 110	2.6± 0.1

PCL number average molecular weights of 340, 760, and 1200 daltons produced PCLF copolymers with molecular weights of 3150, 3680, and 3870 daltons, respectively. For all molecular weights, the polydispersity index of PCLF was significantly higher than that of PCL. The degree of crystallinity of PCLF depended on PCL molecular weight. For example, as the PCL molecular weight increased from 340 to 760 and 1200 Daltons, percent crystallinity of PCLF increased from 9% to 45% and 52%, respectively. The melting point of PCLF, measured by DSC, was 32, 48, and 52C for PCL molecular weights of 340, 760, and 1200 Daltons, respectively. The rate of degradation of PCLF in PBS at 37C depended on the PCL molecular weight. For example, for PCL with Mn of 1200 Daltons, Mw of PCLF decreased from 5.2×10^4 Daltons at time zero to 4.0×10^4 , 2.7×10^4 , 1.8×10^4 , and 1.1×10^4 after 4, 8, 12, and 24 weeks, respectively. Tissue compatibility was evaluated by subcutaneous implantation in Sprague Dawley rats for 4 weeks. The PCLF disk and surrounding tissue were removed and examined histologically by H&E staining. Monoclonal antibody specific to allograft inflammatory factor (AIF-1) was used for immunohistochemistry.

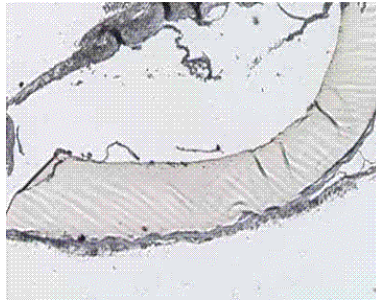


Figure 1

No inflammatory response was observed in the surrounding tissue. The thickness of the fibrous capsule around the sample was less than 100 μm , which was comparable to Gore-Tex as the negative control. The biocompatible PCLF macromer is potentially useful as a self-crosslinkable scaffold for guided tissue regeneration.

Conclusions

A novel self-crosslinkable degradable biomaterial was synthesized by copolymerization of fumaryl chloride with poly(caprolactone). This self-crosslinkable material could serve as a biodegradable and self-crosslinkable scaffold for bone regeneration.

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References

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